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Original Article

A PROSPECTIVE OBSERVATIONAL STUDY OF VITAMIN D3 LEVEL IN REPRODUCTIVE AGE GROUP WOMEN WITH LEIOMYOMA UTERI

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ABSTRACT

Uterine leiomyomas are common benign tumours of the uterus whose pathogenesis remains poorly understood.In India, the incidence is high and it inflicts a heavy burden on women`s health and healthcare system by being a common aetiology for menorrhagia and dysmenorrhea in women of reproductive age group.Incidence is between 5.4 to 77%.Vitamin D deficiency is a proven risk factor in the pathogenesis of uterine fibroid in many studies conducted in different parts of the world but not many studies have been conducted on Indian women.

Methods: A total of 200 women of age group 18 to 45 years attending District Hospital,Howrah,West Bengal, were included in the study.Out of which,100 had leiomyoma and rest healthy women without leiomyoma serves as controls.Routine ultrasound examination and serum hormone analysis of Vitamin D3 were done.Serum FSH(Follicle Stimulating Hormone), LH (Luteinizing Hormone) were done on day 2 of menstruation.Statistical analysis of data was performed using SPSS Version 22 software.

Results: The mean serum concentration of vitamin D3 was significantly lower in women with uterine fibroids compared to controls ($p<0.003$).On further analysis, 68.4% of cases were found to be severely deficient (vitamin D3 <9 ng/ml) as compared to 27.12% of controls ($p<0.0002$).Besides that only 3.67% of cases had sufficient vitamin D level as compared to 22.16% of controls ($P<0.0002$).The Odds Ratio (OR) of occurrence of fibroid with serum Vitamin D3 level of < 12 ng/dl compared to that level > 12 ng/dl was 5.38 ($p<0.0001$).

Conclusion: Serum Vitamin D3 level inversely correlated with the burden of leiomyoma and possibly its deficiency is a causative factor for the occurrence of uterine fibroid in the reproductive age group women.

Keywords: Risk Factor, Uterine fibroid/ leiomyoma, Vitamin D deficiency.

BACKGROUND

Uterine leiomyomas are common benign tumours of the uterus whose pathogenesis remains poorly understood. In India, the incidence is high and it inflicts a heavy burden on women`s health and healthcare system by being a common an etiology for menorrhagia

and dysmenorrhea in women of reproductive age group. Incidence is between 5.4 to 77%[1].

Multifactorial etiopathology with hormonal factors, African-American race, nulliparity, obesity, and a positive history of fibroids are the risk factors for high rate of leiomyoma. They may be asymptomatic or can cause abnormal bleeding, pelvic pressure symptoms, infertility and growth or regress throughout the life[2].

Vitamin D3 deficiency has been suggested to be a risk factor in many chronic conditions like cardiovascular disease, autoimmune disease, and also in several types of cancers[3].Several examples of consistent in vitro and in vivo experimental evidence support in Europe and Africa support its implications in the pathogenesis of uterine fibroid. Three studies showed that both myometrial and leiomyoma cells are highly sensitive to the regulatory effect of 1,25-dihydroxyvitamin D3[4].

The biological effects of vitamin D3 is essentially through its activation of VDR (Vitamin D receptor) cellular receptor, which in turn alters transcription rate of target genes responsible for various biological responses. This includes reduction in cell proliferation and regulation of biological processes including angiogenesis, extracellular matrix production and immune response. In an vivo model of leiomyomas in rats, Halder et al demonstrated that 1, 25 – dihydroxy vitamin D3 causes a dramatic reduction in the dimension of the lesions[5,6].

Hypovitaminosis including vitamin D deficiency is very common in Indian women. To shed more light on the possible role of vitamin D in the development of this pathology, we took up this study to assess serum levels of vitamin D3 in reproductive age group women with and without fibroids.

METHODOLOGY

This study was designed as a cross-sectional observational study after approval of the Institutional ethics committee of District Hospital Howrah, West Bengal, India. (Ref no: HDH/How/IEC/Non-spon/592/06-2019) The study population included women between 18 to 45years of age visiting Obs & Gynae dept. Of District Hospital Howrah from 1st Feb 2019 to 31st Jan 2020.Women with at least one uterine fibroid of >1.5cm^3 in volume or larger in TVS along with serum FSH and LH level <10 mIU/ml measured on day 2 of their menstrual cycle were eligible as cases. Control subjects were recruited from women of similar age group as cases with normal uterus on ultrasound examination. Exclusion criteria were women with history of pregnancy or miscarriage within last 6 months, and women currently on hormonal therapy or vitamin supplements, patients with chronic diseases like hypertension, diabetes, autoimmune disorders, coronary, hepatic, or renal diseases were also excluded from both the groups. A written informed consent was obtained from all patients.

After a brief history and physical examination, all recruited patients underwent TVS (transvaginal sonography) using 6 MHz transvaginal probe. Parameters like uterine size (in three perpendicular planes), number of fibroid lesions, volume of all fibroid lesions (by Prolate Ellipse Formula = a x b x c x 0.523 where a is height, b is width, and c is depth) were noted down. Patints in whom TVS was not sufficient to evaluate fibroid lesion in their entire entity, especially in large sized fibroid, Transabdominal sonography (TAS) was performed. Blood samples were collected from all patients to measure serum FSH and serum vitamin D 25 (OH) D_3 level. Both these quantitative parameters were measured by automated chemiluminescent immunoassay (CLIA) technology.

Data analysis was performed using Statistics Package for Social Sciences version 22.0 (SPSS, Chicago, Illinois). Statistically significant differences were determined using Fisher`s exact test, $X^2$ test, unpaired Wilcoxon test, or Student`s t test, as appropriate. A stepwise forward logistic regression model was used to adjust for variables known to be associated with leiomyomas (body mass index (BMI), black ethnicity, parity). A P value <= 0.5 was considered statistically significant.

The sample size was calculated based on an expected concentration of 25-hydroxyvitamin D_3 in controls of 20.5 +/- 11.3 ng/ml from a previous study, and it was hypothesized that a decrease of 20% of this value in women with uterine fibroid would be clinically significant. Considering alpha error of 5% and power of study 80%, a calculated sample would have been a minimum of 170 patients with 85 patients in each group. However, we included all eligible 100 patients visited to our hospital during the study period along with 100 matching controls.

RESULTS

Baseline parameters of both case and control groups were comparable except a statistically significant higher BMI was noticed in women with fibroid (Table 1). Most of the patients were multiparous in both the groups. Menorrhagia (50%) was the most common presenting complaint followed by pain in abdomen (27.45%) and dysmenorrhea (14.22%) in women with fibroid.

Table 1: Baseline parameters of cases with fibroid and control

Parameters	Cases of fibroid (n = 100)	Controls (n = 100)	p value
Age (years)	40.79 +/- 3.21	42.18 +/- 4.37	0.649
BMI (kg/m2)	28.12 +/- 2.56	27.87 +/- 2.23	0.016
Parity (%)			
P_0	5.78%	5.78%	0.96
P_1	18.44%	14.78%	0.88
P_2	38.89%	42.06%	0.67
>= P_3	34.74%	37.11%	0.78
Age at menarche(years)	13.40 +/- 1.04	13.56 +/- 1.04	0.158
Day 2 serum FSH (IU/ml)	6.48+/- 2.20	6.88+/- 1.03	0.543
Day 2 serum LH (IU/ml)	7.45+/- 3.20	7.89 +/- 1.21	0.512
Demographic distribution			
Urban (%)	70.23%	66.12%	0.756
Rural (%)	29.77%	33.88%	0.675

Serum levels of 25-hydroxyvitamin D_3 were significantly lower in women with fibroids than in controls (Table 2). Severe deficiency of vitamin D_3 were significantly seen in women with fibroids than in controls (Table 2). Severe deficiency of vitamin D_3 (< 10 ng/dl) was noticed in 66% of women with fibroids and 28% in controls. Moreover, only 3.66% of women with fibroids had sufficient (>30 ng/dl) serum levels of vitamin D_3 as compared to 24% in controls. The odds ratio (OR) of occurrence of fibroid with serum vitamin D_3 level of <10 ng/dl compared to that of serum vitamin D_3 level of > 10 ng/dl was 3.56 (95% C.I: 2.11 – 8.94) (p = 0.0001)

Table 2: Serum vitamin D_3 level in cases with fibroid and control

Parameters	Cases of fibroid (n = 100)	Controls (n = 100)	p value
Serum level (ng/dl)	10.27 +/- 5.14	24.45+/- 15.12	<0.0002
Serum vitamin D level categories n (%)			
Severe Deficiency (<10)	50	32	<0.0002
Deficiency (10-20)	30	24	0.67
Insufficient (20-30)	12	24	0.002
Sufficient (>30)	8	20	0.001

Further analysis of cases in terms of fibroid number and size was performed in relation to serum vitamin D_3 level to explore possible association. In women with number of fibroids more than two, the serum vitamin D_3 level was lower in comparison with women with fibroids less than two. However, the result was not statistically significant (8.36 +/- 6.45 ng/dl vs 11.24 +/- 7.22 ng/dl; p = 0.36). We failed to find out any correlation between volume of the largest fibroid and serum vitamin D_3 level.

Table 3 Serum vitamin D_3 and number of fibroids

No. of Fibroids	No. of patients	Level of serum vitamin D (ng/ml) (mean +/- SD)	p value
< 2	70	12.25+/- 6.45	0.29
>= 2	30	7.56+/- 4.77	0.11

DISCUSSION

Our study demonstrated significantly lower serum vitamin D_3 level in women with fibroid as

compared to control population ($p<0.0002$). Furthermore, the relative odd of the presence of fibroid in a woman with vitamin D_3 level <10ng/dl was 5.34 (95% confidence interval (CI) 3.45-8.67) ($p=0.0001$). This finding suggests a possible inverse correlation between serum vitamin D_3 and uterine fibroid in the present study population which corroborates the results of the studies conducted on different populations outside India.

A cross sectional study conducted by Halder et al included 104 women with fibroid and 50 controls without the disease. They similarly reported lower mean serum vitamin D_3 concentration among cases with fibroid. A retrospective analysis of the data by Baird and colleagues reported that women with sufficient vitamin D_3 (>20 ng/dl) had an estimated 32% lower odds of fibroids compared with those with vitamin D insufficiency (adjusted odds ratio 0.68, 95% confidence interval 0.48-0.96)[7,8,9]. So, all these studies conducted in different geographical locations confirm our study hypothesis. However, in contrast to most of these studies, serum vitamin D_3 level is significantly lower with fibroid in our study[10,11].

On categorical analysis of vitamin D levels, we found that 66% of women with fibroid are associated with severe deficiency (<10 ng/dl) as compared to 28% in controls. A similar kind of analysis was performed by Paffoni et al in their study which revealed that 15% of women with fibroid had severe deficiency as compared to 7% in controls and sufficient vitamin D level was found in 37% of cases as compared to 45% in controls. So, the correlation appears to be quite relevant in our study as compared to the study by Paffoni et al.as a greater number of women with fibroid in our study have severe vitamin D_3 deficiency compared to controls[12,13].

The benefit of measuring 25-hydroxyvitamin D_3, for monitoring serum vitamin D level, is that it represents the total body vitamin D from dietary intake, sunlight exposure and peripheral conversion of vitamin D. On the other hand, it has a shorter half-life of 15days.Therefore, its level as a causative factor in the development of fibroid can be erroneous. However, studies found that baseline serum vitamin D_3 level remains stable over long period of time (i.e person with a particular serum level tends to remain constant during multiple years of follow up)[14]

The biggest problem in drawing inferences from a cross-sectional designed studies are confounders. We tried to address some of the risk factors associated with the development of fibroid like age, parity, and BMI by carefully choosing controls. The possibility of reverse causation is also an issue to be dealt with.

Another pertinent question, which needs to be answered, is whether vitamin D deficiency is implicated in the development or growth of the fibroid. Our data did not reveal any significant correlation between the fibroid number and size with serum vitamin D_3 level, which indirectly suggested its role in both development and growth of fibroid. In contrast, Paffoni reported that the vitamin D deficiency correlated more with the number than the size of the fibroid, suggesting its implication more with the development than the growth of fibroid[15,16].

The most suitable approach to fulfill the aim of demonstrating a causal relationship would be a prospective long-lasting cohort study with serial monitoring of vitamin D status and regular follow up of patients as to see how many of them actually develop uterine fibroid. This type of exhaustive analytical study may take many years and difficult to achieve[17,18].

Halder et al reported reduction in size of uterine fibroid in the Eker rat model after vitamin D_3 supplementation. Some in vitro studies have also found a dose-dependent inhibitory effect of vitamin D on human fibroid cell growth. Most of the study demonstrated the effect of 1, 25-dihydroxyvitamin D_3 on apoptosis, modulation of several cell growth genes, protein synthesis, and cell proliferation. These functions are the base of anti-tumor effects of 1,25-dihydroxyvitamin D_3 on leiomyoma[19].

One of the plausible explanations towards fibroid development is altered extracellular matrix production due to aberrant response to tissue repair. Vitamin D might suppress this

abnormal response by regulating the extracellular matrix production. In addition, studies have speculated that it inhibits catechol-O-methyl transferase an enzyme supposed to be overexpressed in human uterine fibroid leading to suppression of growth of fibroid cells[20].

In India dietary deficiency appears to be the primary a etiology of vitamin D deficiency in females. Moreover, the incidence of uterine fibroid in reproductive age group is around 37% as reported by a study in South India. This number in all probabilities might br higher as many more are undetected. So, implication of vitamin D deficiency in uterine fibroid will bring in a whole new therapeutic aspect and it will impart a huge impact in the outcome[21,22].

CONCLUSION

Our study showed a definite indirect association of vitamin D deficiency and uterine fibroid in this part of India. To start supplements at the correct time and spreading awareness among patients should also be given due importance. However, further studies are warranted in this regard.

LIMITATION

This study has some limitations that should be addressed in future studies. Data regarding the extent of sun exposure, measurement of dietary intake of calcium and vitamin D, skin measurement of dietary intake of calcium and vitamin D, skin colour, use of sunscreens and other likely factors that are associated with vitamin D deficiency are not included in this study. Further researches are needed to evaluate these causes of vitamin D deficiency as well before assessing its relation with uterine fibroids in women of reproductive age group.

Compliance with Ethical standards

Conflict of interest Dr Annesha Dutta, Dr Murari Mohan Koley, Dr Preeti Gautam, declare that they have no conflict of interest.

Informed Consent in Studies with Human Subjects All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

REFERENCES

1.Myers ER, Barber MD, Gustilo-Ashby T, Couchman G, Matchar DB, McCrory DC. Management of uterine leiomyomata: what do we really know? Obstet Gynecol. 2002;100:8 –17.

2. Laughlin SK, Schroeder JC, Baird DD. New directions in the epi- demiology of uterine fibroids. Semin Reprod Med. 2010;28:204– 217.

3. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white wom- en: ultrasound evidence. Am J Obstet Gynecol. 2003;188:100 –107.

4. Somigliana E, Vercellini P, Daguati R, Pasin R, De Giorgi O, Cro- signani PG. Fibroids and female reproduction: a critical analysis of the evidence. Hum Reprod Update. 2007;13:465–476.

5. LukJ, TorrealdayS, NealPerryG, PalL.Relevance of vitamin D in reproduction. Hum Reprod. 2012;27:3015–3027.

6. Sabry M, Al-Hendy A. Innovative oral treatments of uterine leio- myoma. Obstet Gynecol Int. 2012:943635.

7. Bläuer M, Rovio PH, Ylikomi T, Heinonen PK. Vitamin D inhibits myometrial and leiomyoma cell proliferation in vitro. Fertil Steril. 2009;91:1919 –1925.

8. Sharan C, Halder SK, Thota C, Jaleel T, Nair S, Al-Hendy A. Vi- tamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. Fertil Steril. 2011;95:247–253.

9. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3 reduces TGF-beta3-induced fibrosis-related gene expression in hu- man uterine leiomyoma cells. J Clin Endocrinol Metab. 2011;96: E754 –E762.

10. Halder SK, Sharan C, Al-Hendy A. 1,25-Dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biol Reprod. 2012;86:116.

11. SabryM,HalderSK,AllahAS,RoshdyE,RajaratnamV, Al-Hendy A. Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: a cross-sectional observational study.

Int J Womens Health. 2013;5:93–100.
12.Khyade RL. A study of menstrual disturbances in cases of fibroid uterus.Int J Reprod Contracept Obstet Gynecol. 2017;6:2494-7.
13.Paffoni A, Somigliana E, Vigano` P, et al. Vitamin D status in women with uterine leiomyomas. J Clin Endocrinol Metab.2013;98(8);E1374-8.
Lippman SA, Warner M, Samuels S, et al. Uterine fibroids and gynecologic pain symptoms in a population- based study. Fertil Steril 2003; 80: 1488-94.
14.Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: a review. Environ Health Perspect 2003; 111: 1037-54.
15. Kjerulff K, Langenberg P, Seidman J, Stolley P, Guzinski G. Uterine leiomyomas: racial differences in severity, symptoms and age at diagnosis. J Reprod Med 1996; 41: 483-90.
16. Munusamy MM, Sheela WG, Lakshmi VP.Clinical presentation and prevalence of uterine fibroids: a 3 year study in 3-decade rural South Indian women. Int J Reprod Contracept Obstet Gynecol. 2017;6:5596-601.
17. Pérez-López FR, Ornat L, Ceausu I, et al. EMAS position statement: management of uterine fibroids. Maturitas 2014; 79; 106-16.
18. Parker WH. Etiology, symptomatology and diagnosis of uterine myomas. Fertil Steril 2007; 87: 725-36.
19.Olive DL, Lindheim SR, Pritts EA. Non-surgical management of leiomyoma: impact on fertility. Curr Opin Obstet Gynecol 2004; 16: 239-43.
20.Sharan C, Halder SK, Thota C, et al. Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-omethyl-transferase. Fertil Steril. 2011;95:247-53.
21.Ylikomi T, Laaksi I, Lou YR, et al. Anti-proliferative action of vitamin D. Vitam Horm. 2002;64:357-406.
22.Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014;6(2): 729-75.

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